National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2389

Amino Acids in 0.1 mol/L Hydrochloric Acid

This Standard Reference Material (SRM) is a solution of 17 amino acids in a 0.1 mol/L aqueous solution of hydrochloric acid. Certified values are provided for all 17 amino acids. This SRM is intended primarily for the use in the calibration of chromatographic instrumentation for the determination of the amino acids. A unit of SRM 2389 consists of five 2-mL ampoules each containing approximately 1.2 mL of the solution.

Certified Concentrations of Amino Acids: The certified concentrations and estimated uncertainties for the 17 amino acids are given in Table 1. These values are based on the results obtained from the NIST analytical determinations using liquid chromatography (LC) and a round robin study comprised of 13 participants conducted with the cooperation of the Association of Biomolecular Research Facilities (ABRF).

Expiration of Certification: The certification of this SRM is valid until **01 January 2010**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in the certificate. However, the certification is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The coordination of the technical measurements leading to the certification was performed in the NIST Organic Analytical Research Division by S.A. Margolis and S.A. Wise. Coordination of stability measurements was performed by D.M. Bunk and M.J. Welch of the NIST Analytical Chemistry Division.

Statistical consultations on the experimental design and the evaluation of the data were provided by S.B. Schiller of the NIST Statistical Engineering Division. Statistical evaluation of the stability data was performed by N.F. Zhang of the NIST Statistical Engineering Division.

The support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

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Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 06 January 2006

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Measurement Services Division

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Table 1. Certified Concentrations of Amino Acids in SRM 2389

Amino Acid	Concentration mmol/L[(a)]
Alanine	2.51 ± 0.09
Arginine	2.94 ± 0.14
Aspartic Acid	2.50 ± 0.09
Cystine	1.16 ± 0.06 [(b)]
Glutamic Acid	2.27 ± 0.10 [(c)]
Glycine	2.45 ± 0.08
Histidine	2.83 ± 0.11
Isoleucine	2.39 ± 0.07
Leucine	2.48 ± 0.09
Lysine	2.47 ± 0.10
Methionine	2.43 ± 0.09
Phenylalanine	2.44 ± 0.08
Proline	2.44 ± 0.09
Serine	2.43 ± 0.09
Threonine	2.39 ± 0.08
Tyrosine	2.47 ± 0.09
Valine	2.44 ± 0.08

[(a)] The certified value is the equally-weighted mean of the NIST average and the round robin average. The expanded uncertainty, U, reported above is two times the combined standard uncertainty, u_c, for the certified value. The combined standard uncertainty for the certified value is one-half the sum, in quadrature, of the NIST and round robin standard uncertainties and conforms to NIST guidelines [1].

[(b)] The uncertainty for cystine was expanded to three times u_c to account for increased uncertainty based upon recent measurements.

[(c)] The certified value and uncertainty for glutamic acid are based upon new measurements at NIST.

NOTICE AND WARNING TO USERS

Handling: This material contains 0.1 mol/L hydrochloric acid and should be handled with care because it is corrosive and may cause burns. Use proper disposal methods.

Storage: Sealed ampoules, as received, should be stored in the dark at approximately 4 °C.

INSTRUCTIONS FOR USE

Sample aliquots should be drawn for analysis at 20 °C to 25 °C immediately after opening the ampoule. The ampoules were sealed under nitrogen and although the amino acids are relatively stable, some long-term degradation may occur.

The round robin study was conducted with the cooperation of the Association of Biomolecular Research Facilities (ABRF). All participants in the study were members of the ABRF and are listed in Appendix 2.

PREPARATION AND ANALYSIS

Preparation of Material: Preparation and analytical determinations were performed in the NIST Organic Analytical Research Division by S.A. Margolis. Stability measurements were performed by D.M. Bunk and M. Vergne of the NIST Analytical Chemistry Division.

NIST LC Measurements: All chemicals used in the preparation of this SRM were obtained from commercial sources. The amino acid solution was prepared by weighing the individual amino acids, concentrated HCl, and water and mixing until the amino acids were completely dissolved. The total mass of this solution was measured. The concentration of each amino acid was calculated using the density of 0.1 mol/L HCl at 23 °C of 0.9990 g/cm^3. The purity of each amino acid was examined by LC analysis for contamination with other amino acids. Arginine, glutamic acid, serine, and threonine contained from 0.2 % to 3.5 % amino acid impurities. The remaining amino acids contained less than 0.25 % of amino acid impurities. The gravimetric values for each amino acid were adjusted to account for these impurities. The amino acids were not examined for other organic or inorganic impurities except for histidine, which was found to be a mixture of the free base and the chloride salt using nuclear magnetic resonance spectroscopy. The bulk solution was dispensed under dry nitrogen in 1.2 mL aliquots into 2-mL amber ampoules which were then flame sealed.

Aliquots from 15 randomly selected ampoules were analyzed in duplicate by LC on an Amino-Tag Column (Varian Analytical Instruments, San Fernando, CA) employing UV detection (at 260 nm) of the precolumn derivatized 9-fluorenylmethyl chloroformate (FMOC) derivative of the amino acids. Three external standards (phenylalanine, tyrosine, and histidine) were used for quantification purposes. The external standards were prepared gravimetrically in 0.1 mol/L KOH (phenylalanine and tyrosine) or water (histidine) at a concentration suitable for measuring their absorbance at 258.2, 298, and 211 nm, respectively [2]. The exact concentration was calculated for each external standard using its molecular absorbance at the indicated wavelength [2]. These solutions were further diluted by weight to prepare four mixtures of the three amino acids at four different concentrations bracketing the concentration of the amino acids in the SRM. These were used to determine the calibration curves. Extensive investigation of the FMOC derivative indicated that all FMOC amino acid derivatives except tyrosine, lysine, and histidine exhibit similar UV molecular absorbances. Similar UV molecular absorbances were also observed for tyrosine, lysine, and histidine which are doubly derivatized by FMOC.

Round Robin Analyses: The participating labortories analyzed two independent samples using the method(s) routinely used in their laboratory. These included a variety of instruments, standardization techniques, and methods. Three basic types of derivatizing agents were used by the laboratories: Ninhydrin (6 laboratories), phenyl isothiocyanate (6 laboratories) or fluorescamine (1 laboratory). Calibration was achieved by using either a commercial mixture of amino acids or an internal standard such as norleucine.

Stability Measurements: Amino acid analyses on a subset of the analytes were performed using two different approaches. Approach one involved isotope dilution liquid chromatography-tandem mass spectrometry. Weighed aliquots of acetonitrile solutions of deuterated analogs of the target amino acids were added to weighed aliquots of the SRM solution. The amino acids were separated using hydrophilic interaction chromatography on a polyhydroxyethyl aspartate column with a gradient mobile phase of 0.2 % formic acid in acetonitrile (mobile phase A) and 0.2 % formic acid in 10 mmol/L ammonium formate in water (mobile phase B). Electrospray ionization was used to generate $(M+H)^+$ ions, which were collisionally dissociated in the triple quadrupole mass spectrometer. Daughter ions at $(M - 45)^+$ were monitored. Known mixtures of pure unlabeled amino acids and their deuterated analogs were used to generate linear calibration plots. Measurements were performed over three days, with duplicate aliquots from each of three vials analyzed each day. Analytes measured using this technique were phenylalanine, methionine, valine, serine, and glutamic acid.

Approach two involved use of a commercial amino acid analyzer employing ion-exchange chromatography with ninhyridin post-column derivation and spectrophotometric detection. Calibration was performed using gravimetrically prepared solutions of the pure amino acids. Measurements were performed over two days, with triplicate injections from one solution each day. Analytes measured using this technique were aspartic acid, cystine, phenylalanine, methionine, valine, serine, and glutamic acid.

SUPPLEMENTAL INFORMATION

Noncertified Quantitative Values

Appendix 1 contains supplementary analytical results and information obtained during the certification of SRM 2389, including (a) the gravimetric value from the preparation of the solution, (b) the results of the NIST LC measurements, and (c) the results of a round robin study on this SRM. Appendix 2 contains a list of the participants in the round robin study.

REFERENCES

[1] Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297, U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs/.

[2] *Handbook of Biochemistry and Molecular Biology*, 3 rd ed., Vol. III, Proteins; Fasman, G.D., Ed.; CRC Press: Cleveland, OH; pp. 186–191 (1976).

Certificate Revision History: 06 January 2006 (This revision reflects a change in the certified value for glutamic acid, expanding the uncertainty for cysteine and an extension of the certification period); 06 December 1993 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; email srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX 1. Summary of the Analytical Results for SRM 2389 [a]

Amino Acid	Amino Acid Concentration [b] mmol/L Gravimetric Value [c]	NIST LC Measurements [d]	Round Robin Study [e]
Alanine	2.50	2.62 ± 0.07	2.39 ± 0.05
Arginine	2.83	3.03 ± 0.11	2.85 ± 0.09
Aspartic Acid	2.55	2.57 ± 0.07	2.44 ± 0.06
Cystine	1.20	1.16 ± 0.06	1.17 ± 0.05
Glutamic Acid	2.44	2.53 ± 0.06	2.41 ± 0.05
Glycine	2.51	2.54 ± 0.07	2.37 ± 0.05
Histidine	2.49	2.77 ± 0.06	2.88 ± 0.09
Isoleucine	2.54	2.36 ± 0.05	2.41 ± 0.05
Leucine	2.60	2.56 ± 0.08	2.40 ± 0.05
Lysine	2.51	2.52 ± 0.08	2.42 ± 0.06
Methionine	2.53	2.48 ± 0.06	2.37 ± 0.06
Phenylalanine	2.58	2.44 ± 0.06	2.44 ± 0.05
Proline	2.50	2.50 ± 0.06	2.37 ± 0.06
Serine	2.47	2.50 ± 0.07	2.35 ± 0.05
Threonine	2.44	2.41 ± 0.06	2.38 ± 0.04
Tyrosine	2.49	2.52 ± 0.08	2.42 ± 0.05
Valine	2.55	2.48 ± 0.06	2.40 ± 0.05

[a] The summary of results given above is presented as supplemental information to the certified values. **The certified values,however, are the best estimates of true concentration for calibration, method validation, and other quality control purposes.**

[b] The amino acid content is based on the molecular weight of the free amino acid except for histidine which is based on the molecular weight of the HCl salt. The histidine is actually a mixture of the free base and the salt which accounts for the discrepancy between the gravimetric and assayed values.

[c] The gravimetric value is based on the weighed amount of each amino acid used to prepare the solution.

[d] This is the mean value for duplicate measurements on 15 discreet samples (n = 30) and the standard uncertainty. To determine the standard uncertainty, a 95 % confidence interval for the average corrected LC peak area was intersected with 95 % confidence bands for the calibration curve. The confidence interval for the average corrected peak area includes between-day as well as within-day variance components for the LC measurements. The standard uncertainty is the 95 % confidence interval for the mean, divided by 2.

[e] The round robin average is the average of the lab means and the standard uncertainty is the standard deviation of that average. N = 26 independent samples except cystine (n = 22) and proline and valine (n = 24).

APPENDIX 2. Laboratories Participating in the Round Robin Study

Stanford University Medical Center	Palo Alto, CA
Pfizer Central Research	Groton, CT
Abbott Laboratories	Abbott Park, IL
Biogen Corporation	Cambridge, MA
Massachusetts Institute of Technology	Cambridge, MA
Ciba-Geigy Biotech	Research Triangle Park, NC
Hoffman-LaRoche Inc.	Nutley, NJ
W. Alton Jones Cell Science Laboratory	Lake Placid, NY
Rockefeller University	New York City, NY
Wistar Institute	Philadelphia, PA
AAA Laboratory	Mercer Island, WA
Medical College of Wisconsin	Milwaukee, WI
Beckman Company	Palo Alto, CA